The Human Glutathione *S*-Transferase Supergene Family, Its Polymorphism, and Its Effects on Susceptibility to Lung Cancer

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Cytosolic glutathione *S*-transferases (GSTs) are a supergene family of dimeric enzymes capable of detoxifying a number of carcinogenic electrophiles. Of the numerous components of tobacco smoke, the polycyclic aromatic hydrocarbons appear to be the principal compounds that yield substrates for these enzymes, GSTM1-1 being effective with those PAH derivatives so far studied; however, the gene locus for GSTM1 is polymorphic, containing two well-characterized expressing genes and a null allele. Use of cDNA for GSTM1-1 or appropriate fragments of genomic clones as probes in Southern blots indicated that the null allele is due to the absence of *GSTM1*. In preliminary experiments, described here, with lung tissue from smokers, levels of ^{32}P-postlabeled nuclease P1-enhanced DNA adducts were inversely correlated with levels of antigen cross-reacting with antibody to GSTM1-1, suggesting that initiation depends on the expression of GSTM1-1. Since similar quantities of DNA adducts and GSTM1-1 activity have been shown to occur in bronchial and peripheral lung, however, the development of malignancy, which is usually in the bronchial region, presumably depends on additional factors that bring about promotion and progression, which are not necessarily affected by *GSTM1* expression. Two epidemiological studies have been carried out in which a possible correlation between the absence of *GSTM1* and lung cancer incidence is considered. In the first, involving a U.S. population sample, smokers with and without lung cancer were phenotyped, and a highly significant correlation between the absence of GSTM1-1 activity and adenocarcinoma of the lung was observed. In the second, involving genotyping of a British population, the correlation between the homozygous *GSTM1* null genotype and lung cancer was much less significant and concerned squamous-cell carcinoma.

Introduction

Considerable effort has been put into studies intended to determine the mechanisms whereby tobacco smoking brings about carcinogenesis in the lung, but much remains to be learned. The activity of enzymes that toxify and detoxify carcinogens known to be present in tobacco smoke has been an important area of study; recently, genetic polymorphisms in both the cytochrome P450 CYP2D6 (debrisoquine hydroxylase) (*1–3*) and one of the glutathione *S*-transferase isoenzymes (EC 2.5.1.1.18) (GSTs), GSTM1 [GSTμ/ψ (*4–6*)], have been implicated in susceptibility to the disease. This paper focuses on the GSTs and examines what is known of their possible roles in pulmonary and extrapulmonary tissues with respect to anticarcinogenesis in human lungs exposed to cigarette smoke.

Glutathione *S*-Transferases and Anticarcinogenesis

GSTs are multifunctional proteins that catalyze many reactions between glutathione (GSH) and lipophilic compounds with electrophilic centers, including those which are cytotoxic and genotoxic. When the reaction of GSH with an electrophile forms a stable covalent bond, the resultant adduct, referred to as a GSH conjugate, is usually no longer toxic and is excreted. All compounds that possess an electrophilic carbon, or acquire one by metabolism, form adducts by spontaneous reaction, at rates that

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vary considerably depending on the nature of the electrophilic center (e.g., the second-order rate constant for the reaction of GSH at pH 7 with the cytotoxin *N*-acetyl-*p*-benzoquinoneimine is 3×10^4 $M^{-1}s^{-1}$, but that for the carcinogen metabolite 1-nitropyrene-4,5-oxide is only 0.17 $M^{-1}s^{-1}$). GSTs catalyze many, but not all, of these reactions. These enzymes also catalyze GSH-dependent reactions that do not form stable adducts, such as hydroperoxide reduction and certain isomerizations, which might also be involved in tobacco smoke carcinogenesis during tumor promotion and tumor progression. These activities are not discussed in the present paper (*7*).

Electrophiles such as benzo[*a*]pyrene (BP)-7,8-diol-9,10-oxide (BPDE), which react with DNA to produce a carcinogenic lesion, have a slow spontaneous rate of reaction with GSH; however, if this rate is sufficiently enhanced by GST catalysis, the resulting reaction competes effectively with DNA adduct formation and GSTs are powerful anticarcinogens (*7*). The importance of GSH conjugation in anticarcinogenesis is illustrated by the case of aflatoxin B_1 (AFB_1) carcinogenesis in rat liver. AFB_1 is metabolized to the electrophile AFB_1-8,9-oxide, which reacts readily with DNA but is poorly conjugated at normal GST levels in the rat liver, with the result that AFB_1 is a powerful rat hepatocarcinogen. Inducers of GST expression reduce AFB_1 binding by DNA to very low levels, and AFB_1-induced carcinogenesis is all but abolished (*8*). So far, evidence as direct as this is not available for other carcinogens: The effect of GSTs on carcinogenesis is usually inferred from the known enzymic activities of purified enzymes and knowledge of their cellular concentrations.

Glutathione *S*-Transferase Supergene Family

In rats and humans, numerous soluble GSTs have been identified and shown to be dimers, the subunits of which are members of a supergene family composed of four multigene families, referred to as α, μ, π, and θ. The most extensively studied GSTs are those of the rat, in which five α, five μ, one π, and two θ class GST subunits have been identified by the combined efforts of a number of laboratories (*7*). As work progresses, the existence of a similar multiplicity of forms is becoming apparent among the human enzymes. The nomenclature is at present very confusing, but a rational system based on published guidelines for human gene nomenclature (*9*), similar to that used for the cytochrome P450 supergene family, was devised recently and agreed upon by many of the principal workers in the field. This system is used in the present paper (Table 1).

Many of the biologically significant substrates for GSTs are difficult to synthesize, are labile, and are not ideal for enzymic assay, with the result that the supply of data is limited. Table 2 shows the values that have been obtained for BP-4,5-oxide (BPO), the GSH conjugate of which is a major excretory product of BP in the rat (*10*) and perhaps also in man; +(*anti*)-BPDE, which is the ultimate carcinogenic electrophile derived from metabolism of BP; and *trans*-stilbene oxide, which is used in studies of GSTM1-1 polymorphisms (*7,24*).

Distribution of Glutathione *S*-Transferase Isoenzymes in Human Lung and Liver

Tissues vary considerably in overall GST concentration and also have very specific distributions of GST subunits. Such quantitative and qualitative differences between tissues are presumably important in determining the susceptibility of a tissue to a carcinogen. Tables 3 and 4 address the situation seen in man, in whom the individual differences can be very great (*7*). Table 3 shows quantitative values obtained by high-performance liquid chromatography (HPLC), using the method of Ostlund Farrants et al. (*25*), for expression of GST subunits A1, A2, and M1 in a number of human liver samples (*26*). The absence of GST subunit M1 in a number of cases is noteworthy. It will be seen below that the GSTM1 null phenotype occurs in about 50% of the population. Table 4 shows the results of quantitative western blotting [for method, see Towbin et al. (*27*)] of 11 resected samples of human lung. Preliminary HPLC analyses indicated that A1 and A2 are detected in the α class GST subunits M1 and M3 in the μ class GST subunits, and P1 in the π class GST subunit.

Table 1. Nomenclature for human glutathione *S*-transferases (GSTs).[a]

Proteins				Genes		
Previous designation for GST	Class	New designation	References	Locus designation	Chromosome	References
ϵ, B_1B_1, GST2-type 1, H_a (subunit 1), $\alpha_x\alpha_x$	α	GSTA1-1	(*11–13*)	*GSTA1*	6	(*12*)
δ, B_2B_2, GST2-type 2, H_a (subunit 2), $\alpha_y\alpha_y$	α	GSTA2-2	(*13,14*)	*GSTA2*	6	(*12*)
μ, GST1-type 2, H_b (subunit 4)	μ	GSTM1a-1a	(*15*)	*GSTM1*	1	(*15*)
ψ, GST1-type 1	μ	GSTM1b-1b	(*4*)	*GSTM1*	1	(*15*)
Muscle, GST4	μ	GSTM2-2	(*16*)	*GSTM2*		
Brain, GST5	μ	GSTM3-3	(*17*)	*GSTM3*		
π, GST3	π	GSTP1-1	(*18,19*)	*GSTP1*	11	(*20*)
GST θ[b]	θ		(*21*)			
Microsomal GST	Microsomal		(*22*)	*GST12*	12	(*23*)

[a] From B. Mannervik, University of Uppsala, Uppsala, Sweden.

[b] Available data on amino acid sequence demonstrate unambiguously the existence of a distinct class named θ, but until complete primary structure is known it will not be given a designation in the new system (*21*).

Table 2. Activities of human glutathione transferases (GSTs) toward several substrates of importance.

GST	BPO	BPDE	TSO
Containing subunits GSTA1 and/or GSTA2	0.05	ND	0.002
GSTM1-1	0.92	0.69	5.2
GSTM2-2	ND	ND	ND
GSTM3-3	ND	ND	ND
GSTP1-1	0.13	2.9	0.002

Abbreviations: BPO, benzo[*a*]pyrene-4,5-oxide (*7,24*); BPDE, (+)*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (*7*); TSO, *trans*-stilbene-oxide (*24*); ND, not determined.

Figures 1*A* and 1*B* show for comparison the relative GST contents of samples of human liver and lung as demonstrated by HPLC separations. Figure 1*C* shows the HPLC analysis of sufficient of the lung GST protein shown in Figure 1*B* to indicate the details of its subunit composition.

Lung Tissue and Lung Cancer

The two lobes of the lung begin at the end of the trachea, which divides into two bronchi; each of these extends and further divides into bronchioles, which undergo increasing ramification, ending in the terminal bronchioles, which open into the mass of alveoli. Proximally, the pseudostratified epithelium of the bronchi is lined with flattened ciliated cells, together with mucus-secreting goblet cells. Proceeding along the bronchiolar tree, the larger bronchioles have a simple columnar epithelium, which is also ciliated, while the smaller bronchioles have a cuboidal epithelium lacking cilia. Finally, in the alveoli, the principal cells are types I and II pneumocytes, the former providing

Table 3. Variation in glutathione *S*-transferase (GST) subunit and debrisoquine 4-hydroxylase content of human liver.[a]

	GST subunit, μg/g liver			Debrisoquine 4-hydroxylase, nmole/mg microsomal
Sample	A1	A2	M1	protein
HL15	94	32	–	0.19
HL16	76	33	–	0.06
HL19	252	37	19	–
HL23	187	25	–	0.09
FH39	300	300	–	0.01
FH41	118	99	–	0.08
FH47	32	150	14	0.05
FH50	149	191	3	0.07
FH56	102	125	42	0.05
FH61	85	385	–	0.03
FH77	404	81	–	0.08
FH80	156	289	–	0.15
FH81	266	277	–	0.10
FH82	93	37	–	0.08
FH83	551	146	–	0.09
FH55	16	47	28	0.01
HL49	99	83	6	–
FH70	76	92	21	–
HL25	125	53	–	–
HL62	131	130	14	–
FH84	169	94	–	–
HL91	364	333	–	–
Mean ± SEM	174.7 ± 28.2	138.1 ± 23.4	18.4 ± 4.4	0.076 ± 0.012

[a]Data from Ketterer et al. (*26*).

Table 4. Glutathione *S*-transferase (GST) content of human adult lung tissue.[a]

GST subunit, ng/mg pulmonary protein		
A1 + A2	M1 + M3	P1
95	24	273
37	62	190
115	97	431
96	174	312
51	nil	273
65	nil	132
41	81	1431
115	327	2117
232	207	2914
19	nil	174
23	19	137
80.8 ± 17.6[b]	89.3 ± 27.8	762 ± 27.9

[a]Normal tissue obtained from lung cancer patients; GST subunits were obtained by quantitative Western blotting (*27*). The titer for GST subunit M3 is much lower than that for GST subunit M1.
[b]Mean ± SEM.

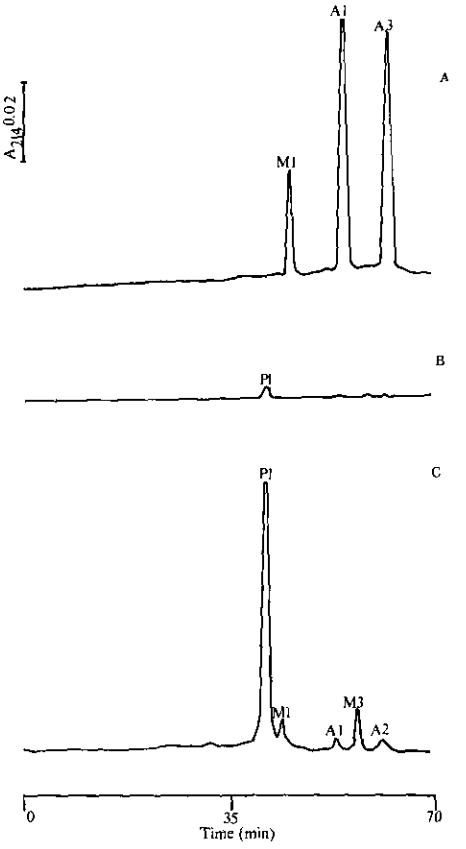

FIGURE 1. Glutathione *S*-transferase (GST) subunit content of samples of human liver and lung. (*A,B*) chromatograms obtained using 300 μg of tissue protein and (*C*) a chromatogram obtained using 13.6 mg of the same lung tissue as shown in Figure 1*B*. GSTs were analyzed by affinity chromatography followed by high-performance liquid chromatography according to Ostlund Farrants et al. (*25*).

most of the alveolar surface, as they are remarkably thin in transverse section, and the principal site of gaseous exchange with the pulmonary capillaries. The vascularization of the lung in this region is more extensive than that in any other part of the body. Type I cells are capable of the active pinocytosis of particles that reach their surface, resulting in their transfer to the interstitium. Both the interstitium and the lumen of the alveolus contain macrophages which engulf microorganisms and other particulates, such as those derived from tobacco smoke. Those that remain in the interstitium contribute to the dark lung color seen in smokers, while those that enter the alveolar lumen (millions per day) pass up and out of the lung by mucociliary action of the bronchi and upper bronchioles (*28,29*).

These are the major cellular components of the lung, but minor components should not be overlooked, such as the neuroepithelial APUD (amine uptake and decarboxylation) cells which accumulate and secrete biogenic amines and are believed to be the cells of origin of small-cell lung carcinoma. Cells of origin for squamous-cell carcinoma and adenocarcinoma, which are the most frequently occurring lung cancers, and of the rarer large-cell carcinomas have not been identified. It is important to note that all these cancers usually occur in the more proximal parts of the lung—hence the term bronchiogenic carcinoma. Both squamous-cell and globlet-cell metaplasia have been observed in the bronchi of smokers, but whether or not these are preneoplastic lesions has not been ascertained (*30*).

Activation of Carcinogens in the Lung

Although cancer is mostly associated with the bronchial region, it cannot be assumed that most carcinogens are removed and activated in the upper regions of the lungs and either do not reach the alveoli in sufficient quantity to be dangerous or are very effectively detoxified there. Levels of activating enzymes, such as aryl hydrocarbon hydroxylase and epoxide hydrolase, are present in similar amounts in bronchial and alveolar tissue in smokers (*31*), and ^{32}P-postlabeling studies on bronchial and alveolar tissues from lungs of smokers show that the two regions of the lung have similar levels of DNA adducts (*32*).

Bronchoalveolar macrophages are also active in xenobiotic metabolism and contain aryl hydrocarbon hydroxylase, epoxide hydrolase, and GST. The macrophages engulf smoke particulates, which carry adsorbed carcinogens such as polycyclic aromatic hydrocarbons (PAH) and are capable of metabolizing them (*33,34*). The principal function of these macrophages is to produce bacteriocidal (and cytotoxic) oxidizing agents in response to ingested bacteria; however, other particulates, such as those found in tobacco smoke, also activate the cytotoxic activity of macrophages. Tobacco smoke is also toxic to the cilated epithelium, greatly reducing the effectiveness of mucociliary clearance of the lung. It is possible that, as a result, the rate of passage of macrophages through the bronchi is reduced and that their cytotoxins, together with some of the carcinogenic metabolites they produce, have the opportunity to affect the bronchial epithelium (*35*).

Glutathione *S*-Transferases of Normal and Smokers' Lungs

Table 4 shows the GST composition of samples of lung tissue from a number of adults. In agreement with the work of Di Ilio et al. (*36*) and Fryer et al. (*37*), who separated native enzymes, GSTP1-1 is the major component. Ontogenetic studies have shown that GSTP1-1 is very dominant at the earliest stages, when the primitive lung is an invagination of the endoderm and undergoing the branching associated with the growing bronchial tree. As development proceeds, the level falls steeply and then remains stable during the rest of fetal life, the postnatal period, and adult life (the lung is vascularized early in fetal life). The α class enzymes occur at low levels throughout all stages of development, and GSTM1-1 occurs at even lower levels in those individuals in whom it is expressed. The studies in which these findings were made were done before GSTM3-3 and other members of the μ family (apart from GSTM1-1) had been isolated and identified.

Preparations of bronchial and peripheral tissue from adults have similar BPO-GST activities (*31*). Smoking causes a small but significant depression in BPO-GST activity in lung tissue overall (*38*), but bronchiolar macrophages undergo a considerable reduction (*34*).

Relationship between Level of DNA Adducts and Glutathione *S*-Transferase Content of the Lungs of Smokers

Although the properties and tissue distribution of GSTs have been described, it is unclear how they might affect detoxication of the many components of tobacco smoke that have been shown to be carcinogenic and that may be involved in the etiology of smoking-induced lung cancer. A list of some of the carcinogens known to be present in cigarette smoke includes PAH, nitrosamines, and aromatic amines (*35*). So far, only PAH metabolites have been shown to be substrates for GSTs. Thus, the assays for BPO-GST activity described above are relevant for humans (and are believed to be important in rats) (*10*), to the extent that BPO-GSH conjugation is an important pathway of BP detoxication. BPO is known to be a good substrate for GSTM1-1, less so for GSTP1-1, and even less so for GSTs containing A1 and A2. (+) *anti*-BPDE, however, which is known to be the ultimate carcinogen in some tissues (e.g., skin), is a good substrate for GSTM1-1, and an even better one for GSTP1-1, which not only results in higher rates than other GSTs but is more selective for the metabolically produced (+) *anti*-enantiomer (*7*). Unfortunately, no data are available on GSTM3-3 and other μ-class enzymes. Since the GSTs M1-1 and P1-1 are known to catalyze the detoxication of PAH *in vitro*, one might expect to see some effect of GSTs on PAH–DNA adduct levels in smokers. Figure 2 shows a comparison between the levels of nuclease P1-enhanced ^{32}P-postlabeled DNA adducts [see the method described in Talaska et al. (*39*)] and of GST subunits M1 + M3 in smokers and nonsmokers. These are preliminary results; nevertheless, there appears to be an

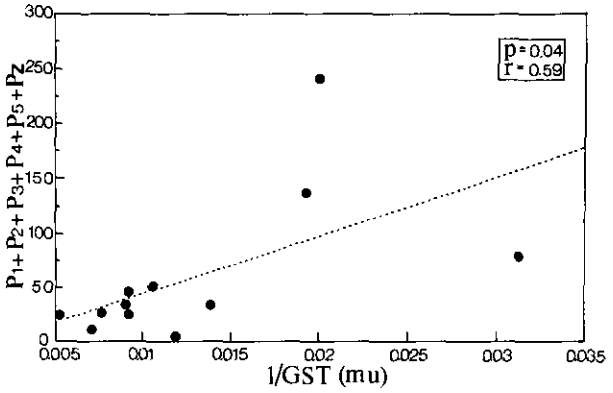

FIGURE 2. Relationship in the lungs of smokers between levels of nuclease P1-enhanced ^{32}P-postlabeled DNA adducts and of antigen cross-reacting with the antibody to GSTM1-1 (which may also have a low level of cross-reactivity with GSTM3-3). The method for ^{32}P-postlabeling and quantitative immunodetection using ^{131}I-labeled antibody are described by Towbin et al. (*27*) and Talaska et al. (*39*), respectively.

inverse correlation. No correlation was seen with GST subunits A1 + A2 or P1.

A proportion of the smoke that enters the lungs is taken up by the epithelium during its passage down the bronchiolar tree, but the remainder gains ready access to the bloodstream in the alveoli. Figure 1 shows that the detoxication capacity of the lung is very much lower than that of the liver and perhaps of other extrapulmonary tissues. The same is true of the activating enzymes (*30*). The consequence is that tobacco smoke produces DNA adducts not only in lung tissue but also in a number of extrahepatic tissues, ranging from those most immediately accessible, such as the heart, to those more distant, such as the liver and kidney (*40*). Table 2 shows that little effect of GSTs containing subunits A1 and/or A2 is to be expected; even the major component GSTP1-1 occurs at low levels compared to the levels of GSTs found in the liver. Products derived from tobacco that reach the blood may recirculate and be further metabolized by the lung, but their amount will be considerably reduced and they will be changed qualitatively after passage through the liver. It may be that the adducts that are found in the lung depend not only on the metabolism of inhaled aerosol entering tissue from the lumen but also on the activation of progenotoxins (e.g., BP-7,8-diol, 9-OH-BP) entering lung tissue from the bloodstream. Levels of extrapulmonary PAH and PAH derivatives may be affected profoundly by whether or not GSTM1-1 is expressed in the liver, since cytochrome P450s are inhibited by the products of their action. GST and epoxide hydrolase have important functions in removing these inhibitors (*10*). The results shown in Table 3 might then be the result of the combined effects of primary and secondary pulmonary metabolism, the latter being most important in the peripheral lung where the blood supply is so rich. It should be noted, however, that, regardless of their origin, the levels of DNA adducts are similar in the bronchial and peripheral regions of the lung although lung cancer is much more common in the bronchi.

Relationship between Lung Cancer and Glutathione *S*-Transferase Genotype

Evidence has been given that DNA adduct formation is inversely correlated with the GST μ-class phenotype and there may be a similar correlation with susceptibility to lung cancer. Thus, Seidegard and his colleagues (*4,5,24*) have demonstrated a significant correlation between the occurrence of carcinoma of the lung and low levels of *trans*-stilbene oxide-GSH transferase activity in blood leukocytes, this compound having been shown to be a particularly good substrate for GSTM1-1 (Table 2). They compared smokers with and without lung cancer and found that the GSTM1 null phenotype was associated with susceptibility to lung cancer and that this association was highly significant in patients with adenocarcinoma (Table 5). Using Southern blots of DNA obtained from blood cells, probed with subunit GSTM1b cDNA, they compared phenotype with genotype in a handful of cases and showed that the GSTM1 null phenotype was associated with a deletion; thus, in the *GSTM1*O/GSTM1*O* genotype, the *GSTM1* gene is partly or totally absent (*5*).

In this particular population sample, studied by phenotyping, there was a preponderance of adenocarcinomas, which is regarded as not typical. Squamous-cell carcinomas are the commonest among smokers in western society, although adenocarcinomas appear to be on the increase (*30*). In a recent study by Zhong et al. (*6*), involving genotyping rather than phenotyping, in which probes were derived from highly conserved regions of μ family genomic DNA (*41*), the correlation between *GSTM1*0/GSTM1*0* and adenocarcinoma was, if anything, negative. There was, however, a small but positive correlation of *GSTM1*O/GSTM1*O* with squamous-cell carcinoma. This population had the more usual preponderance of squamous cell carcinomas (Table 6) (*30*). The discrepancy between the results of these two studies is not yet understood.

Some μ-class genes are clustered. The remarkable conservation in genomic structure in both exons and regions of introns which has been shown in this family shows that gene conversion has occurred and should continue to do so (*41*). A situation can be envisaged in which a gene conversion gives a positive genotype but a null phenotype, because a conversion event has occurred, and results in a negative regulatory effect on the gene. Such a

Table 5. Association between risk for lung cancer among smokers and the GSTM1 null phenotype.[a]

Subjects	GSTM1-1/GSTM1 null	*p*-Value
Smokers		
Small-cell carcinoma	3/12	0.02
Large-cell carcinoma	4/10	0.25
Squamous-cell carcinoma	27/62	0.04
Adenocarcinoma	28/93	0.0001
Other	8/14	0.93
Total	70/191	0.001
Controls	112/192	

[a]Data from Seidegard et al. (*5*).

Table 6. Proportion of *GSTM1*0/GSTM1*0* in individuals with and without lung cancer.[a]

Genotype	All controls (225)	All lung cancers[b] (228)	Squamous-cell carcinoma[c] (100)	Adenocarcinoma[d] (56)	Other lung cancers[e] (72)
*GSTM1*0/GSTM1*0*	58% (131)	57% (130)	52% (52)	29% (16)	58% (42)
GSTM1 detected	42% (94)	43% (98)	48% (48)	71% (40)	42% (30)

[a]Data from Zhong et al. (*6*); figures in parentheses indicate numbers of individuals tested in each category.
[b]Controls/all lung cancers: $\psi^2 = 0.07$; $p = 0.85$.
[c]Controls/squamous-cell carcinomas: $\psi^2 = 2.92$; $p = 0.09$.
[d]Controls/adenocarcinomas: $\psi^2 = 3.28$; $p = 0.07$.
[e]Controls/other lung cancers: $\psi^2 = 0.02$; $p = 0.99$.

hybrid would not have the *GSTM1*O*-associated deletion or ever give rise to a *GSTM1*O/GSTM1*O* genotype; however, such gene conversions would not be of sufficient frequency to explain the discrepancy between the results of Seidegard et al. (*5*) and those of Zhong et al (*6*), although they could explain occasional inconsistencies between genotype and phenotype. One should also be aware that genotyping by polymerase chain reaction of members of the GST μ multigene family, which has such a high level of sequence identity in introns and exons arising from gene conversion, may result in an artefactually positive attribution of genotype.

Discussion

The lung has the capacity to activate and detoxify BP, and presumably other PAHs, in both the bronchiolar and alveolar epithelia (*31*), but the levels of many activating and detoxifying enzymes in this organ are low overall, and a significant proportion of the PAH load that a tobacco smoker encounters may pass into the systemic blood to be toxified and detoxified in extrapulmonary tissues (*40*). Among the members of the GST supergene family for which the specificity toward some substrates is known, GSTM1-1 is effective with both a presumed major epoxide metabolite of BP, namely BPO, and the ultimate carcinogen BPDE (*7*) and is therefore important for their detoxication and excretion. (The activities of the GSTs M2-2 and M3-3 with these substrates have yet to be determined, and the activities towards other electrophilic substrates such as BP-7,8-oxide are not known.) Even so, the importance of the μ family for the detoxication of PAH is borne out by our preliminary experiments in which the level of immunochemically determined μ family GSTs in the lungs of smokers appears to be inversely correlated with its nuclease-P1 ^{32}P-postlabeled DNA adducts. The *GSTM1* locus is polymorphic and has a null allele that occurs at such frequency that *GSTM1* null phenotypes are present in appoximately 50% of the population (*4–6,40*). Individuals with null phenotypes are expected to have reduced abilities to detoxify BPO and BPDE. Thus, the expression of GSTM1-1 could affect the development of PAH-dependent cancer; this should be apparent when either the GSTM1-1 phenotype or the *GSTM1* genotype is determined.

It should be noted that, in the two studies performed so far, the analysis of phenotype has shown much more significant correlations than studies of the genotype (*4–6*). This might be due to the different epidemiological designs of the two studies, to the different origins of the two populations studied (the phenotyping having been done on a U.S. group and the genotyping on a British group), or to other undetermined factors. It should be borne in mind that at least one other genetic polymorphism is associated with lung cancer, namely that involving the debrisoquine hydroxylase (CYP2D6) locus (*1–3*). There is no linkage with the *GSTM1* polymorphism, with the result that the effect of one may either obscure or enhance the effects of the other or be neutral, according to inheritance. Simultaneous genotyping of the same individuals for the GSTM1 deletion and CYP2D6 mutants might reveal populations with very significant differences in susceptibility to lung cancer. A glance at Table 3 shows individuals in which GSTM1 and debrisoquine hydroxylase expression might work with each other (HL15, FH80) or against each other (FH55) to promote susceptibility to lung cancer.

Since the levels of GSTs, including GST subunit M1, in the lung are low (Fig. 1), a significant proportion of the detoxication of PAH from tobacco smoke may occur in the extrapulmonary tissue and some DNA damage in the lung may be secondary, due to bloodborne progenotoxic PAH metabolites released from other organs.

Data currently available do not explain why lung tumors are mostly associated with the bronchi rather than with the alveoli, to which PAH are similarly accessible. Unknown but critical promotion events may occur in the bronchus which do not occur more distally. In this respect, bronchoalveolar macrophages may play a role: not only are their levels of BPO-GST sharply reduced by smoking (*34*), but they may also spend more time in the bronchial area of smokers, since mucociliary action is inhibited by tobacco smoke (*35*). Wherever they are, they produce not only PAH metabolites but also cytotoxic active oxygen, which is a promoter in the skin and perhaps also in the lung [see also Kadlubar et al. (*42*)].

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REFERENCES

1. Ayesh, R., Idle, J. R., Ritchie, J. C., Crothers, M. J., and Hetzel, M. R. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. Nature 312: 169–170 (1984).

2. Caporoso, N. E., Tucker, M. A., Hoover, R. N., Hayes, R. B., Pickle, L. W., Issaq, H. J., Muschik, G. M., Green-Gallo, L., Buivys, D., Aisner, S., Resau, J. H., Trump, B. F., Tollerud, D., Weston, A., and Harris, C. C. Lung cancer and the debrisoquine metabolic phenotype. J. Natl. Cancer. Inst. 82: 1264–1271 (1990).
3. Gough, A. C., Miles, J. S., Spurr, N. K., Moss, J. E., Gaedigk, A., Eichelbaum, M., and Wolf, C. R. Identification of the primary gene defect of the cytochrome P450 *CYP2D* locus. Nature 347: 773–776 (1990).
4. Seidegard, J., Vorachek, W. R., Pero, R. W., and Pearson, W. R. Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. Proc. Natl. Acad. Sci. USA 85: 7293–7297 (1988).
5. Seidegard, J., Pero, R. W., Markowitz, M. M., Roush, G., Miller, D. G., and Beattie, E. J. Isoenzyme(s) of glutathione transferase (class mu) as a marker for the susceptibility to lung cancer: a follow up study. Carcinogenesis 11: 33–36 (1990).
6. Zhong, S., Howie, A. F., Ketterer, B., Taylor, J., Hayes, J. D., Beckett, G. J., Wathen, C. G., Wolf, C. R., and Spurr, N. K. Glutathione S-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. Carcinogenesis 12: 1533–1537 (1991).
7. Ketterer, B., Meyer, D. J., and Clark, A. G. Soluble glutathione transferase isozymes. In: Glutathione Conjugation (H. Sies and B. Ketterer, Eds.), Academic Press, London, 1988, pp. 73–135.
8. Kensler, T. W., Egner, P. A., Davidson, N. E., Roebuck, B. D. Pickul, A., and Groopman, J. D. Modulation of aflatoxin metabolism, aflatoxin-N^7-guanyl formation and hepatic tumorigenesis in rats fed ethoxyquin: role of induction of glutathione S-transferases. Cancer Res. 46: 3924–3931 (1986).
9. Shows, T. B., McAlpine, P. J., Bouchiex, C., Collins, F. S., Conneally, P. M., Frezal, J., Gershowitz, H., Goodfellow, P. N., Hall, J. G., Issit, P., Jones, C. A., Knowles, B. B., Lewis, M., McKusick, V. A., Meisler, M., Morton, N. E., Rubinstein, P., Schanfield, M. S., Schmickel, R. D., Skolnick, M. H., Spence, M. A., Sutherland, G. R., Traver, M., Van Cong, N., and Willard, H. F. Guidelines for human gene nomenclature. An international system for human gene nomenclature. Cytogenet. Cell Genet. 46: 11–18 (1987).
10. Cooper, C. S., Grover, P. L., and Sims, P. The metabolism and activation of benzo[a]pyrene. In: Progress in Drug Metabolism, Vol. 7 (J. W. Bridges and L. F. Chasseaud, Eds.), John Wiley and Sons, New York, 1983, pp. 295–396.
11. Tu, C.-P. D., and Qian, B. Human liver glutathione S-transferases: complete primary sequences of an Ha subunit cDNA. Biochem. Biophys. Res. Commun. 141: 229–237 (1986).
12. Board, P. G., and Webb, G. C. Isolation of a cDNA clone and localisation of human glutathione S-transferase 2 genes to chromosome band 6p12. Proc. Natl. Acad. Sci. USA 84: 2377–2381 (1987).
13. Hayes, J. D., Kerr, L. A., and Cronshaw, A. D. Evidence that glutathione S-transferases B_1B_1 and B_2B_2 are the products of separate genes and that their expression in human liver is subject to inter-individual variation. Biochem. J. 264: 437–445 (1989).
14. Rhoads, D. M., Zarlengo, R. P., and Tu, C.-P. D. The basic glutathione S-transferases from human livers are products of separate genes. Biochem. Biophys. Res. Commun. 145: 424–481 (1987).
15. DeJong, J. L., Chang, C.-M., Whang-Peng, J., Knutson, T., and Tu, C.-P. D. The human liver glutathione S-transferase gene superfamily: expression and chromosome mapping of an Hb subunit cDNA. Nucleic Acids Res. 16: 8541–8554 (1988).
16. Vorachek, W. R., Pearson, W. R., and Rule, G. S. Cloning expression and characterization of a class mu glutathione transferase from human muscle, the product of the GST4 locus. Proc. Natl. Acad. Sci. USA 88: 4443–4447 (1991).
17. Campbell, E., Takahasi, Y., Abramovitz, M., Peretz, M., and Listowsky, I. A distinct human testis and brain μ-class glutathione S-transferase. J. Biol. Chem. 265: 9188–9193 (1990).
18. Kano, T., Sakai, M., and Muramatsu, M. Structure and expression of a human class π glutathione S-transferase messenger RNA. Cancer Res. 47: 5626–5630 (1987).
19. Ahmad, H., Wilson, D. E., Fritz, R. R., Singh, S. V., Medh, R. D., Nagle, G. T., Awasthi, Y. C., and Kurosky, A. Primary and secondary structural analyses of glutathione S-transferase pi from human placenta. Arch. Biochem. Biophys. 278: 398–408 (1990).
20. Board, P. G., Webb, G. C., and Coggan, M., Isolation of a cDNA clone and localization of the human glutathione S-transferase 3 genes to chromosome bands 11q12 and 12q13-14. Ann. Hum. Genet. 53: 205–213 (1989).
21. Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M., and Ketterer, B. Theta, a new class of glutathione transferase purified from rat and man. Biochem. J. 274: 409–414 (1990).
22. DeJong, J. L., Morgenstern, R., Jornvall, H., DePierre, J. W., and Tu, C.-P. D. Gene expression of rat and human microsomal glutathione S-transferases. J. Biol. Chem. 263: 8430–8436 (1988).
23. DeJong, J. L., Mohandas, T., and Tu, C.-P. D. The gene for the microsomal glutathione S-transferase is on human chromosome 12. Genomics 6: 379–382 (1990).
24. Dostal, L. A., Guthenberg, C., Mannervik, B., and Bend, J. R. Stereoselectivity and regio-selectivity of purified human glutathione S-transferases π, α-ε, μ with alkene and polycyclic arene oxide substrates. Drug Metab. Dispos. 16: 420–424 (1988).
25. Ostlund Farrants, A.-K., Meyer, D. J., Coles, B., Southan, C., Aitken, A., Johnson, P. J., and Ketterer, B. The separation of glutathione transferase subunits by using reverse-phase high-pressure liquid chromatography. Biochem. J. 245: 423–428 (1987).
26. Ketterer, B., Meyer, D. J., Lalor, E., Johnson, P., Guengerich, F. P., Distlerath, L. M., Reilly, P. E. B., Kadlubar, F. F., Flammang, T. J., Yamazoe, Y., and Beaune, P. H. A comparison of levels of glutathione transferases, cytochromes P450 and acetyltransferases in human livers. Biochem. Pharmacol. 41: 635–638 (1991).
27. Towbin, H., Staehelin, T., and Gordon, J. Electophoretic transfer from proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76: 4350–4354 (1979).
28. Ham, A. W., and Cormack, D. H. Histology. J.B. Lippincott, New York, 1979.
29. Hoidal, J. R., and Niewoehner, D. E. Lung phagocyte recruitment and metabolic alterations induced by cigarette smoke in humans and in hamsters. Am. Rev. Respir. Dis. 126: 548–552 (1982).
30. Souhami, R., and Tobias, J. Cancer and Its Management. Blackwell Scientific Publications, Oxford, 1986.
31. Petruzzelli, S., De Flora, S., Bagnasco, M., Hietanen, E., Camus, A.-M., Saracci, R., Izzotti, A., Bartsch, H., and Giuntini, C. Carcinogen metabolism studies in human bronchial and lung parenchymal tissues. Am. Rev. Respir. Dis. 140: 417–422 (1989).
32. Dunn, B. P., Vidal, S., San, R. H. C., Kwan, W.-F., Nelems, B., Emerson, D. A., and Stich, H. F. DNA adducts in bronchial biopsies. Int. J. Cancer 48: 485–492 (1991).
33. Autrup, H., Harris, C. C., Stoner, G. D., Selkirk, J. K., Schafer, P. W., and Trump, B. F. Metabolism of [^{3}H]benzo(a)pyrene by cultured human bronchus pulmonary alveolar macrophages. Lab. Invest. 38: 217–224 (1978).
34. Petruzelli, S., Bernard, P., Paoletti, P., Rane, A., Giuntini, C., and Pacifici, G. M. Presence of epoxide hydrolase and glutathione S-transferase in human pulmonary alveolar macrophages. Eur. J. Clin. Pharmacol. 34: 419–421 (1988).
35. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 38. Tobacco Smoking. International Agency for Research on Cancer, Lyon, 1986.
36. Di Ilio, C., Del Boccio, G., Aceto, A., Casaccia, R., Mucilli, F., and Federici, G. Elevation of glutathione transferase activity in human lung tumor. Carcinogenesis 9: 335-340 (1988).
37. Fryer, A. A., Hume, R., and Strange, R. C. The development of glutathione S-transferase and glutathione peroxidase activities in human lung. Biochim. Biophys. Acta 883: 448-453 (1986).
38. Petruzzelli, S., Camus, A.-M., Carrozzi, L., Ghelarducci, L., Rindi, M., Menconi, G., Angeletti, C. A., Ahotupa, M., Heitanen, E., Aitio, A., Saracci, R., Bartsch, H., and Giuntini, C. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. Cancer Res. 48: 4695–4700 (1988).
39. Talaska, G., Al-Juburi, A. Z. S. S., and Kadlubar, F. F. Smoking related carcinogen–DNA adducts in biopsy samples of human urinary bladder: identification of N-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. Proc. Natl. Acad. Sci. USA 88: 5350–5354 (1991).
40. Randerath, E., Miller, R. H., McHal, D., Avitts, T. A., Dunsford, H. A., and Randerath, K. Covalent DNA damage in tissues of cigarette smokers as determined by ^{32}P-postlabeling assay. J. Natl. Cancer Inst. 81: 341-347 (1989).

41. Taylor, J. B., Oliver, J., Sherrington, R., and Pemble, S. E. Structure of human glutathione S-transferase class mu genes. Biochem. J. 274: 587–593 (1991).

42. Kadlubar, F. F., Butler, M. A., Kaderlik, K. R., Chou, H.-C., and Lang, N. P. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. Environ. Health Perspect. 98: 69–74 (1992).